Characterization of the Major Susceptibility Region for Psoriasis at Chromosome 6p21.3

Nalini Balendran,*[1] R. Lee Clough,†[1] J. Rafael Arguello,‡ Ruth Barber,† Colin Veal,† Andrew B. Jones,§ Jane L. Rosbotham,§ Anne-Margaret Little,‡ Alejandro Madrigal,‡ Jonathan N. W. N. Barker,§ Stephen H. Powis,* and Richard C. Trembath†

*Center for Nephrology, Royal Free and University College Medical School of University College London, Royal Free Campus, London, U.K.; †Division of Medical Genetics, Department of Medicine and Genetics, University of Leicester, Leicester, U.K.; ‡Anthony Nolan Research Institute, The Royal Free Hospital, London, U.K.;
§St Johns Institute of Dermatology, King's College, St Thomas' Hospital, London, U.K.

Psoriasis is a common inflammatory skin condition caused by genetic and environmental factors. Recent genome-wide linkage analyses have identified a locus encoding susceptibility to psoriasis and placed this gene in the 12 cM interval between markers D6S426 and D6S276 on chromosome 6p21.3. This is a broad region and encompasses the human major histocompatibility complex. We have sought to localize the susceptibility gene more precisely by exploiting the linkage, haplotype, and linkage disequilibrium information available through genotyping 118 affected sib pairs, their parents and other affected family members. A total of 14 highly polymorphic markers were genotyped, combining anonymous loci with the class I genes HLA-B and -C distributed across a genetic interval of approximately 14 cM including the entire major histocompatibility complex. Through the application of higher density mapping within the major histocompatibility complex, we identified those regions most commonly shared identical by descent in patients with psoriasis. Using the transmission–disequilibrium test, we found significant evidence of linkage and allelic association across an interval defined by the markers tn62 ($p = 1.0 \times 10^{-7}$), HLA-B ($p = 4.0 \times 10^{-7}$), and HLA-C ($p = 2.7 \times 10^{-9}$), a region encompassed within a 285 kb genomic DNA fragment. Hence these studies contribute to the refinement of the localization of a major psoriasis susceptibility gene and place the critical region near to HLA-C. ***Key words: human leukocyte antigen/linkage/major histocompatibility complex. J Invest Dermatol 113:322–328, 1999***

Psoriasis is a relapsing chronic inflammatory skin disease affecting all racial groups with a peak prevalence of 3% in northern European and Scandinavian caucasians (Lomholt, 1963; Camp, 1998). Phenotypic expression is mainly confined to the skin, with well circumscribed erythematous scaling plaques in a symmetrical distribution. Characteristic features include epidermal keratinocyte hyperproliferation, altered epidermal maturation, vascular proliferation, and inflammatory cell accumulation (Camp, 1998). Whereas the pathogenesis of psoriasis remains uncertain, current evidence suggests a central role for T lymphocytes in modulating the disease process (Barker, 1991).

Epidemiologic studies have implicated a genetic component to psoriasis, including a monozygotic twin concordance rate of 70% compared with a dizygotic twin concordance rate of 20% (Brandrup *et al*, 1982), a 5–10-fold increased risk in first-degree relatives (Theeuwes and Leder, 1993) and large multiple case pedigrees. Previous case–control studies have demonstrated associations with multiple major histocompatibility complex (MHC) class I and II alleles. Interpretation of such studies, however, has been potentially confounded by patient ascertainment bias, population stratification effects, and reliance on serologically defined HLA typing. Genome-wide linkage analyses have identified putative susceptibility loci on chromosomes 1q, 2p, 4q, 6p, 8q, 16q, 17q, and 20p (Tomfohrde *et al*, 1994; Matthews *et al*, 1996; Nair *et al*, 1997; Trembath *et al*, 1997; Capon *et al*, 1999). Recently, two genome wide linkage scans (Nair *et al*, 1997; Trembath *et al*, 1997) and a region specific analysis (Burden *et al*, 1998) have mapped a major locus for psoriasis susceptibility to a 12 cM region at chromosome 6p21.3. As this interval contains the 4 Mb MHC, these studies support prior case–control studies in strongly suggesting that a MHC gene confers the major genetic component to psoriasis.

The human MHC (HLA region) was first identified because it contains genes encoding the major transplantation antigens. The physiologic role for these cell surface molecules, the class I and II antigens, were subsequently shown to be presentation of processed antigen to T lymphocytes (Browning and McMichael, 1996). The MHC is now known to contain over 100 other genes, some of which play additional important parts in the immune response. These observations are of particular interest because immune mediators have been implicated in the pathogenesis of psoriasis

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Reprint requests to: Dr. Stephen H. Powis, Center for Nephrology, Royal Free and University College Medical School, Royal Free Campus, Rowland Hill Street, Hampstead, London NW3 2PF, U.K.
E-mail: powis@rfhsm.ac.uk

Abbreviations: LD, linkage disequilibrium; TDT, transmission-disequilibrium test.

[1]These two authors contributed equally to the paper.

Table I. Characteristics of the study cohort[a]

Affected relations and families	Number
Total number of independent families	78
Total affected sibling pairs	118
2 siblings	54
3 siblings	14
4 siblings	2
5 siblings	1
2 parents available	83
1 parent available	25
0 parents available	10
Total affected relative pairs (non sib)	18
Total number of trios with both parents available	146
Total number of "trios" with one parent available	38
Number of fully independent trios	77
Total number of affected individuals	238
Total number of unaffected individuals	187

[a]In the statistical analyses dependence of multiple affected sibling pairs was taken into account as previously described (Satsangi *et al*, 1996).

(Barker, 1991). As the majority of MHC genes do not have immune function, however, it remains possible that the MHC encoded psoriasis susceptibility gene is not an immune system gene.

Linkage disequilibrium (LD) mapping offers an approach to narrow the MHC candidate region (Spielman and Ewens, 1996). When a mutation or polymorphism responsible for a specific disease is first introduced into a population, it resides on a background haplotype of linked markers in LD with one another. Recombination through many generations will tend to dissipate LD for all but those markers in very close proximity to the disease locus. We now describe the refinement of the MHC candidate region by typing both microsatellite and HLA markers at intermediate density in a British Caucasian psoriasis population. Using allele sharing and family-based linkage disequilibrium analytical methods, we show that a 285 kb region around markers tn62, HLA-B, and HLA-C is most likely to contain the MHC-encoded psoriasis susceptibility gene.

MATERIALS AND METHODS

Family ascertainment Individuals were studied from the caucasoid cohort reported by Trembath *et al* (1997). In brief a total of 78 north European families, ranging from nuclear pedigrees to extended kindreds, all with a minimum of two affected members with psoriasis, were investigated (**Table I**). A diagnosis of psoriasis was made using standard clinical criteria (Camp, 1998). All affected and unaffected individuals were assessed by one of two experienced dermatologists to ensure consistency of application of diagnostic criteria. All probands had chronic plaque type psoriasis and any doubtful cases were excluded. The population consisted of 238 affected individuals, 45% male, 55% female, with a mean age of onset of 16.1 y (range 1–59). Affected individuals were investigated for both allele sharing and linkage disequilibrium. Genomic DNA was extracted from peripheral blood by standard procedures and stored at –70°C until genotyped. Ethical approval was obtained for this study.

Sequence-specific oligonucleotide probing HLA-B genotypes were initially determined by sequence specific oligonucleotide typing as previously described (Yoshida *et al*, 1992). Briefly, four primers (supplied by Oswel DNA Services, Southampton, U.K.) were used for locus-specific amplification of all of exon 2 and all of exon 3. PCR reactions were performed with 1600 ng DNA in an 80 μl reaction containing 8 μl 10 × PCR buffer (Bioline, London, U.K.), 0.195 mM of each primer, 0.2 mM of each dNTP, 2 mM of magnesium chloride, and 1.6 units of *Taq* polymerase (Bioline). Polymerase chain reaction (PCR) amplification was performed as follows: 96°C for 5 min; 96°C for 1 min, 65°C for 1 min, 72°C for 2 min, for 5 cycles; 96°C for 1 min, 55°C for 1 min, 72°C for 2 min, for 25 cycles; 72°C for 8 min (using a 9600 thermal cycler, Perkin-Elmer, Warrington, U.K.). The PCR products were dot blotted, cross-linked on to a nylon membrane and hybridized with 35 digoxigenin labeled sequence-specific oligonucleotide probes (obtained from the British Society for Histocompatibility and Immunogenetics). Following stringent washing, detection was achieved using anti-digoxigenin alkaline phosphatase Fab fragments (Boehringer Mannheim GmbH, Germany). HLA-C typing was performed as previously described (Trembath *et al*, 1997). The nomenclature used for genotyped alleles is the name of the locus followed by an asterisk and the number of the allele (e.g., B*5701); the nomenclature for serologic assignment is the name of the locus followed by the specificity (e.g., B57).

Reference strand mediated conformational analysis Allele-specific high resolution HLA-B genotyping was determined by reference strand mediated conformational analysis (previously described as double strand conformational analysis; Arguello *et al*, 1998). Briefly, fragments containing exon 2, intron 2, and exon 3 of HLA-B were amplified with locus specific primers. Labeled reference strands were prepared from homozygous B-LCLs SP0010 (B*4402) and RSH (B*4201) using 5′ fluorochrome Cy-5 (Pharmacia Biotech, Uppsala, Sweden). Duplexes were formed by the addition of 1 μl of labeled reference PCR product to 3 μl of sample PCR product followed by denaturation at 95°C for 4 min; 55°C for 5 min and addition of an aliquot of each marker mix (2 μl). Duplexes (2 μl) were separated by electrophoresis for 530 min at 40°C through a nondenaturing 6% polyacrylamide gel (Long Ranger Gel Solution, JT Baker, Lichfield, U.K.) in an ALFexpress automated sequencer (Pharmacia) at 30 W constant power. The mobilities of each fluorescent duplex were analyzed using Fragment Manager software (Pharmacia).

Microsatellite genotyping Genotyping was performed for 12 microsatellite polymorphic markers from chromosome 6p21.3. Oligonucleotide primers for microsatellites lh1, d3a, f91, tn821, and tn62 (Lako *et al*, 1999) were kindly provided by Dr R.D. Campbell and amplified as described by Hsieh *et al* (1997). Other microsatellites were chosen form published genetic maps (Reed *et al*, 1994; Dib *et al*, 1996; Martin *et al*, 1998). The CEPH-Genethon map was used to establish intermarker distances for some markers, and the rest were integrated using published physical distances assuming 1 cM ≈ 1 Mb. The mean heterozygosity of markers was 75.1%. Genotypes at each marker were determined using fluorescence labeled 5′ primers and semiautomated techniques as described previously (Trembath *et al*, 1997). Briefly, pooled amplified DNA was electrophoresed on 6% acrylamide gels, which were run for 3 h at 900 V in an ABI 373A automated sequencer (Perkin-Elmer). Semi-automated fragment sizing was performed using GENESCAN 672 (version 2.1) software (Perkin-Elmer), and genotyping carried out using GENOTYPER (version 2.0) software (Perkin-Elmer). The microsatellite RING3CA was genotyped by radionucleotide inclusion according to standard protocols. All genetic marker data were maintained in the Cyrillic database system (Cherwell Scientific, Oxford, U.K.).

Family haplotype analysis Haplotypes were constructed through the application of the Cyrillic pedigree data program and by visual inspection, assuming the minimum number of recombination events observed in each family. If required to resolve apparent multiple recombination events, markers were re-genotyped. All paternity issues had previously been identified and excluded from the study. At each marker locus, an ancestral allele was defined as that allele occurring in greatest excess frequency in the affected cohort compared with the unaffected cohort. The assumed ancestral haplotype, presumed to carry the disease mutation, was then constructed using these alleles (Feder *et al*, 1996).

Statistical analysis Sib pair analysis, requiring no prior assumptions of the mode of inheritance of susceptibility alleles in psoriasis, was performed for the whole cohort using the SIBPAIR program (version 2.1) within the ANALYZE package and as previously described (Trembath *et al*, 1997). The transmission of alleles from heterozygous parents to affected offspring was assessed by the use of the transmission-disequilibrium test (TDT) as implemented in the program TDTLIKE (version 2.1), generating a TDT-like likelihood ratio statistic, based on the algorithm by Terwilliger (1995). Multipoint nonparametric analysis was performed using GENEHUNTER version 1.1. in the nonparametric mode without partitioning for heterogeneity (Kruglyak *et al*, 1996). The program was configured for scoring of all possible affected relative pairs (settings were skip large = off, analysis = nonparametric linkage, score = all, and increment = 5).

RESULTS

Evidence for a psoriasis susceptibility gene located within the interval between markers tn821 and HLA-C As psoriasis is a multifactorial disease with an ambiguous mode of inheritance, nonparametric methods of data analysis, which identify regions of excess allele sharing compared with that under independent assortment, have advantages over conventional parametric linkage

Table II. Linkage results for the study cohort

Marker	No. of alleles observed in cohort	Heterozygosity	SIBPAIR /ASP[a] linkage values		NPL[b] score
			LOD	p	
D6S291	6	0.70	1.20	9.4×10^{-3}	1.78
Ring3ca	10	0.77	2.86	1.4×10^{-4}	1.88
lh1	13	0.71	3.15	7.0×10^{-5}	2.51
d3a	13	0.76	2.07	1.0×10^{-3}	2.75
f9n1	9	0.53	1.63	3.0×10^{-3}	3.06
D6S273	8	0.75	2.45	3.9×10^{-4}	3.28
tn821	13	0.81	3.42	3.6×10^{-5}	3.29
TNFA	15	0.76	5.70	1.5×10^{-7}	3.09
tn62	12	0.82	6.47	2.4×10^{-8}	3.21
HLA B	40	[c]	6.16	5.0×10^{-8}	2.94
HLA C	20	[d]	3.65	2.0×10^{-4}	2.24
D6S265	8	0.78	2.17	9.9×10^{-5}	2.19
D6S105	10	0.79	0.21	1.7×10^{-1}	1.29
D6S276	14	0.83	1.84	1.25×10^{-3}	1.48

[a]ASP, affected sib pair.
[b]NPL, non parametric linkage values obtained from the GENEHUNTER analysis.
[c]223 B alleles defined to date.
[d]63 C alleles defined to date.

analysis. To determine the extent of allele sharing at chromosome 6p21.3, we genotyped 78 independent psoriatic families, containing 118 affected sib pairs and 18 non-sib relative pairs (**Table I**). We analyzed a total of 14 markers (average intermarker distance 0.6 cM) to maximize the information content for the region. LOD scores suggestive of linkage (> 2.4) were maintained across the interval flanked by D6S291 and D6S265 (7.5 cM centromeric to telomeric). Within this interval, evidence of linkage was identified at marker loci tn821 (LOD = 3.42), TNFA (LOD = 5.70), tn62 (LOD = 6.47), HLA-B (LOD = 6.16) and HLA-C (LOD = 3.65). These results are summarized in **Table II**.

Linkage of psoriasis to chromosome 6p21.3 was also demonstrated by analysis of the genotype data with a nonparametric multipoint method, GENEHUNTER, which supported excess allele sharing for the region encompassed by d3a to HLA-B (**Fig 1**) with a maximum nonparametric linkage value of 3.29 for the microsatellite number tn821. For the multipoint analysis we based marker order on the Genethon map and where necessary on known physical intervals, using sex averaged recombination fractions as follows: tel-D6S276–1.0–D6S105–0.5–D6S265–0.4–HLA-C-0.10–HLA-B–0.1–tn62–0.05–TNFA–0.05–tn82–0.1–D6S273–0.1–f9n1–0.35–d3a–0.3–lh1–0.5–Ring3ca–5.45–D6S291–cen.

Linkage disequilibrium (allelic association) analysis To reduce the misleading effects of population stratification on association, non-transmitted parental genotypes were used as internal controls in the TDT as a test of allelic association. For each locus, the alleles with the most significant TDT values (using the multiallelic statistic corrected for multiple allele testing) are represented in **Table III**. Ancestral alleles, as defined as the allele in greatest excess in the patient group compared with the control group, were D6S291–4, Ring3ca–3, lh1–8, d3a–5, f9n1–4, D6S273–2, tn821–11, TNFA-4, tn62–3, HLA-B*5701, HLA-Cw*0602, D6S265–2, D6S105–4, and D6S276–2. Alleles at contiguous loci tn62, HLA-B, and HLA-C, encompassing an $\approx$ 285 kb region, show the strongest linkage disequilibrium with the disease phenotype, all at a significance level of $p < 2 \times 10^{-7}$ using the TDT test. Parent to offspring transmission of alleles HLA-Cw*0602 and HLA-B*5701 occurred on 92 and 60 occasions, respectively, compared with nontransmissions 28 and 16 times, respectively. Immediately flanking markers on the centromeric side of this peak also demonstrate significant allelic association (TNFA, tn821, f9n1), but support for allelic association declines rapidly for more telometric markers (D6S265, D6S105; see **Fig 1**). These data strongly suggest the localization of the susceptibility locus to the proximal part of the MHC in the 285 kb region flanked by markers tn62 and HLA-C.

Haplotype analysis Using the marker order shown above, haplotypes were constructed by inference from the genotypes of the affected, the parents and other family members. Phase assignment was such as to minimize the number of recombination events in each family. Both chromosomes of all affected and unaffected individuals were included in the construction of haplotype sets, giving a total of 422, 130, and 194 chromosomes analyzed for affected, unaffected sibs, and unaffected spouses, respectively. Within these sets, the most common haplotypes are represented in **Table IV**. In the affected individuals the most commonly occurring haplotype, tn62–3, HLA-B*5701, HLA-Cw*0602, includes 91 chromosomes. Although extending the haplotype reduces the total number of chromosomes for the common haplotype, the more extended haplotype f9n1–4, D6S273–5, tn821–11, TNFA-4, tn62–3, HLA-B*5701, HLA-Cw*0602, D6S265–2 more closely resembles the assumed ancestral haplotype. The "fractured" haplotypes are most likely to reflect the effects of recombination, thereby reducing the interval maintained under linkage disequilibrium. For the haplotype f9n1–4, D6S273–5, tn821–11, TNFA-4, tn62–3, HLA-B*5701, HLA-Cw*0602, D6S265–2, the most frequently occurring allele, HLA-Cw*0602, was found in 37% of chromosomes in affected individuals (typically in a heterozygous form) compared with 21% and 18% of chromosomes in unaffected sibs and spouses, respectively. The only other haplotype at greater frequency in the affected cohort also included HLA-Cw*0602. The extended haplotype D6S273–2, tn821–4, TNFA-9, tn62–7, HLA-B*1302, HLA-Cw*0602 occurred in 3%, 1.5%, and 1.3% of all chromosomes in affected individuals, unaffected sibs, and unaffected spouses, respectively. No other common haplotype was observed in the affected group, even for those alleles with significant TDT values as listed in **Table III**.

DISCUSSION

Recent studies of the cosegregation of psoriasis with genome-wide markers by linkage analysis has revealed putative susceptibility loci on chromosomes 1q, 2p, 4q, 6p, 8q, 16q, 17q, and 20p (Tomfohrde *et al*, 1994; Matthews *et al*, 1996; Nair *et al*, 1997; Trembath *et al*, 1997; Capon *et al*, 1999). The most consistent and quantitatively strongest locus conferring susceptibility to psoriasis, however, is 6p21.3, identified in two independent studies using large cohorts of predominantly Caucasian origin (LOD scores > 3; Nair *et al*, 1997; Trembath *et al*, 1997) and confirmed by Burden *et al* (1998).

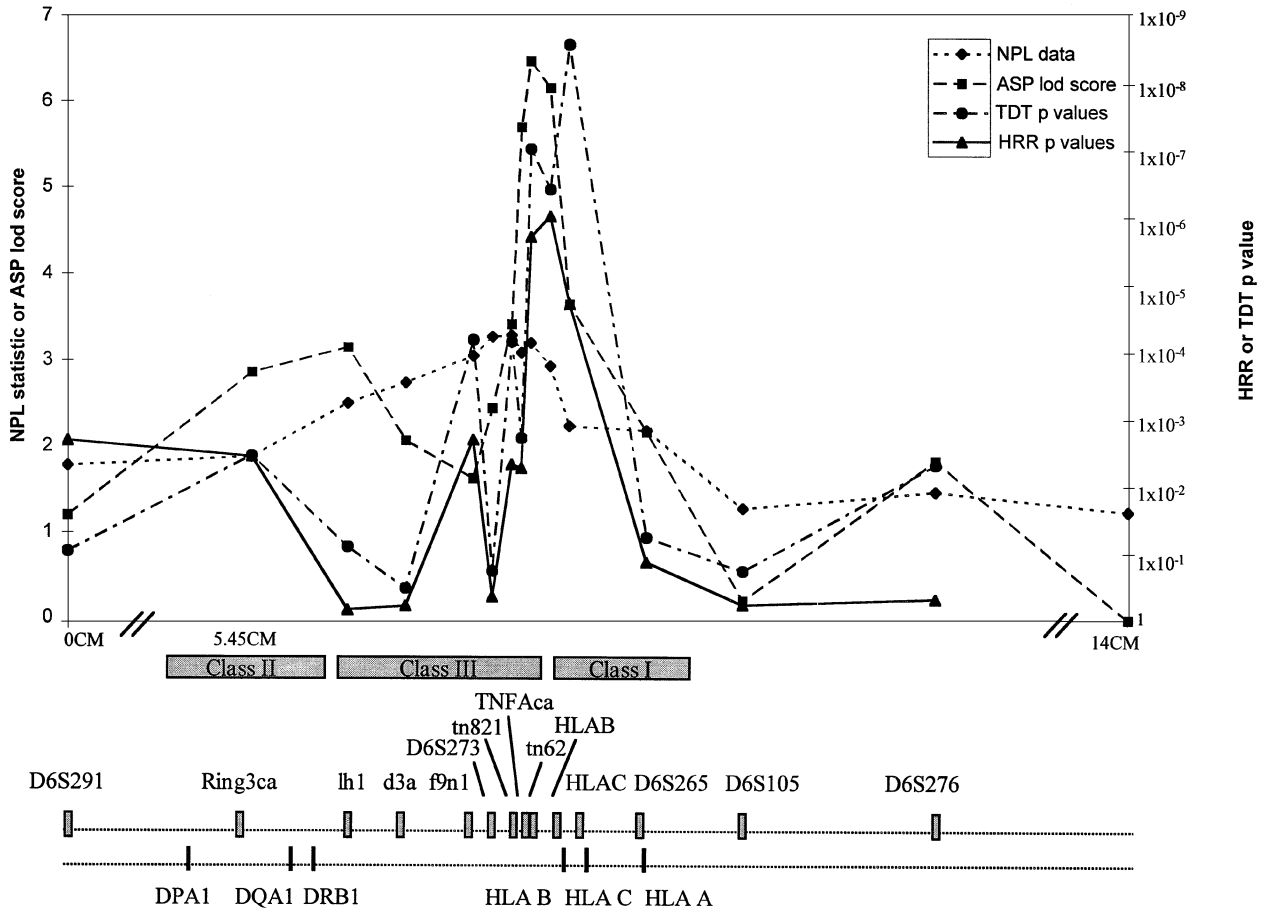

Figure 1. Linkage and association results for the study cohort demonstrating a convergent peak at the boundary of the class III and class I regions. The upper map illustrates genetic distances for the 14 markers used; the lower map physical relationships of known HLA genes in the area.

The 6p21.3 locus was estimated to contribute to greater than 35% of overall familial risk (Trembath *et al*, 1997).

Genome-wide scans necessarily use markers spaced typically at between 10 and 20 cM intervals. Hence our previous study was only able to refine the disease loci to a minimum of a 10 cM range. In this report we have used 14 highly informative polymorphic markers located within the MHC region to localize further the psoriasis susceptibility disease locus. LOD scores significant for linkage (LOD $>$ 3.8) were obtained for three markers TNFA, tn62, and HLA-B ($p < 1.5 \times 10^{-7}$, $p < 2.4 \times 10^{-8}$, $p < 5 \times 10^{-8}$, respectively) within a 250 kbp distance by SIBPAIR allele sharing analysis. Further analysis of the data using GENEHUNTER demonstrated excess allele sharing for a similar but broader region encompassing these three markers. LD mapping, which performs an association analysis minimizing the effects of population stratification, demonstrated significant association for contiguous markers tn62, HLA-B, and HLA-C over a 285 kb region using the TDT. Thus, whereas GENEHUNTER identifies a maximum allele sharing around markers tn82–1 to HLA-B, the TDT indicates the susceptibility region is marginally telomeric to this, between markers tn62 and HLA-C. Therefore, the chromosome 6p21.3 susceptibility locus for psoriasis is likely to be within the interval defined by tn62, HLA-B, and HLA-C, the marker loci exhibiting the most significant allelic associations, at the boundary of the HLA class III and class I regions.

The HLA-B and HLA-C loci, as well as other HLA loci, have previously been associated with susceptibility to psoriasis in numerous case–control studies (Russell *et al*, 1972; White *et al*, 1972; Svejgaard *et al*, 1974; Brenner *et al*, 1978; Tiwari and Terasaki, 1985). The most reproducible associations have been with the class I alleles HLA-Cw6 and HLA-B57. The TDT data obtained in our study have identified the same HLA risk alleles for a Caucasian population. In addition, compared with case–control studies, which usually compare disease populations with unrelated control populations, our study design reduced the potential confounding effects of patient ascertainment bias, small study numbers and population stratification. The use of family-based linkage disequilibrium mapping with internal controls has allowed the analysis of allelic associations independent of population structure (Spielman *et al*, 1993; Schaid and Sommer, 1994; Thomson, 1995). Genotyping rather than the traditional serologic assignment provided more direct evidence that HLA-B*5701 and HLA-Cw*0602 are the alleles strongly associated with psoriasis.

The use of family data in our study permitted the assignment of marker allele phase and thus the unambiguous construction of haplotypes for each individual. The most commonly shared MHC regions "identical by descent" could then be determined. Haplotype analysis demonstrated complete linkage disequilibrium between HLA-B*5701 and HLA-Cw*0602 with no recombination observed between either alleles in any family analyzed. The most common haplotype observed in those affected was tn62–3, HLA-B*5701, HLA-Cw*0602 (91 *vs* 13 $p < 0.0001$ sibs). A second haplotype containing HLA-Cw*0602 was also noted more frequently in affecteds than unaffecteds, however: tn62–7, HLA-B*1302, HLA-Cw*0602 (23 *vs* 3 sibs $p < 0.001$). As HLA-Cw*0602 was the single allele common to both haplotypes these findings would be compatible with three possible explanations. First, that HLA-Cw*0602 is the psoriasis disease susceptibility allele; secondly that HLA-Cw*0602 is in strong linkage disequilibrium with the disease allele. A third possibility is that neither HLA-B nor HLA-C are the disease loci, but that both haplotypes HLA-B*5701-HLA-Cw*0602 and HLA-B*1302-HLA-Cw*0602 define HLA regions

Table III. Association results for the study cohort[a]

Marker	Allele[b]	Allele size[c]	HRR (p value)	Overall TDT (max likelihood estimate) (p value)	Most significant alleles detected via the multiallelic TDT	
					Allele	Allele-specific p value
D6S291	4	207	2.2×10^{-3}	9.7×10^{-2}	4	4.90×10^{-2}
	3	05			3	2.86×10^{2}
Ring3ca	3	–	3.8×10^{-3}	3.7×10^{-3}	3	1.10×10^{-2}
lh1	8	93	7.0×10^{-1}	8.3×10^{-2}	8	2.15×10^{-1}
d3a	5	125	6.5×10^{-1}	3.5×10^{-1}	5	3.37×10^{-1}
f9n1	4	98	2.1×10^{-3}	6.8×10^{-5}	4	2.29×10^{4}
	6	102			6	3.30×10^{1}
D6S273	2	132	4.7×10^{1}	1.9×10^{-1}	2	1.53×10^{-1}
	1	130			1	1.70×10^{-1}
	5	136			5	7.20×10^{-1}
tn821	11	110	5.0×10^{-3}	7.2×10^{-5}	11	2.28×10^{-4}
	3	94			3	8.57×10^{-4}
TNFA	4	104	5.6×10^{-3}	2.0×10^{-3}	4	$8{,}52 \times 10^{-3}$
	7	110			7	7.28×10^{-1}
tn62	3	152	2.0×10^{-6}	1.0×10^{-7}	3	5.07×10^{-7}
	7	160			7	2.37×10^{-1}
HLA-B	5701	–	$1.0 \ 10^{-6}$	4.0×10^{-7}	5701	3.08×10^{-6}
	5001	–			5001	8.97×10^{-2}
HLA-C	0602	–	2.0×10^{-5}	2.7×10^{-9}	0602	2.07×10^{-8}
	1201,12021,12022	–			1201,12021,12022	
D6S265	2	183	1.4×10^{-1}	6.0×10^{-2}	2	1.49×10^{-1}
	6	191			6	2.86×10^{-1}
D6S105	4	153	6.2×10^{-1}	1.9×10^{-1}	2.86×10^{-1}	4.73×10^{-1}
	6	157			6	8.94×10^{-1}
D6S276	2	206	5.0×10^{-1}	5.0×10^{-3}	2	9.42×10^{-3}
	5	214			5	7.55×10^{-1}

[a]The most significant alleles at each locus detected via the multiallelic TDT linkage disequilibrium test (corrected for multiple testing; Spielman and Ewens, 1996) are shown.
[b]HLA-B and-C alleles are defined according to the XII Histocompatibility Workshop nomenclature (Bodmer *et al*, 1996); all other alleles are defined on the basis of their mobility score (Reed *et al*, 1994).
[c]Expressed as mobility units on an ABI 373.

Table IV. Haplotypes associated with susceptibility to psoriasis[a]

(*a*)

f9n1	D6S273	tn821	TNFA	tn62	HLA-B	HLA-C	D6S265	Affected (422 chromosomes)		Unaffected sibs (130 chromosomes)		Unaffected spouses (194 chromosomes)	
								Cumulative haplotypes	Exclusive haplotypes	Cumulative haplotypes	Exclusive haplotypes	Cumulative haplotypes	Exclusive haplotypes
4	5	11	4	3	5701	0602	2	21	21	3	3	3	3
4	5	11	4	3	5701	0602		30	9	3	0	6	3
	5	11	4	3	5701	0602	2	33	12	4	1	5	2
	5	11	4	3	5701	0602		57	15	8	5	10	4
		11	4	3	5701	0602		66	9	10	2	11	1
			4	3	5701	0602		72	6	10	0	14	3
				3	5701	0602		91	19	13	3	17	3
					5701	0602		108	17	16	3	22	5
					5701			125	17	16	0	24	2
						0602		178	70	28	12	42	20

(*b*)

D6S273	tn821	TNFa	tn62	HLA-B	HLA-C	Affecteds (422 chromosomes)		Unaffected sibs (130 chromosomes)		Unaffected spouses (194 chromosomes)	
						Cumulative haplotypes	Exclusive haplotypes	Cumulative haplotypes	Exclusive haplotypes	Cumulative haplotypes	Exclusive haplotypes
2	4	9	7	1302	0602	15		2		3	
	4	9	7	1302	0602	16	1	2	0	3	0
		9	7	1302	0602	17	1	2	0	4	1
			7	1302	0602	23	6	3	1	5	1
				1302	0602	25	2	3	0	7	2
					0602	178	70	28	12	42	20

[a]All unambiguous haplotypes occurring at greater frequency in affected than unaffected individuals are shown. Inferred haplotypes, where data for any of the markers was missing, were excluded from the haplotype subtotals. The most extended haplotypes more closely resemble the "founder" haplotype. The shorter haplotypes reflect the effects of recombination narrowing the region maintained under LD. Exclusive haplotypes represent chromosomes in which a shorter haplotype occurs and has not already been represented within a larger haplotype.

containing alleles at a number of loci which are required in combination for disease predisposition.

In a previous case–control association study, Schmitt-Egenolf *et al* (1996) demonstrated that the extended ancestral haplotype EH57.1, which contains HLA-Cw6 and HLA-B57, conferred a 26-fold increased risk of developing psoriasis, a risk mainly conferred by the class I portion of the haplotype. The relative risk for Cw6-B57 was 26.7 compared with 2.8 for Cw6-B13. The authors suggested that if HLA-C is the disease locus, HLA-B57 may act to modify disease expression. Recently, Jenisch *et al* (1998) also observed a high frequency of the same ancestral haplotype in affected individuals from two large family cohorts. They similarly found that in contrast to the class II region markers HLA-DR and -DQ, the class I markers HLA-C and -B were retained selectively among affected individuals four times more often than among those unaffecteds. As they studied no markers in the 1 Mb region between HLA-DQ and HLA-B, however, they were unable to map the putative disease locus within this region. In contrast, our study analyzed five markers in this region to refine the susceptibility region to 285 kb around the class III/I region border.

HLA-B*5701 and HLA-Cw*0602 have also been associated with psoriasis in Japanese, Indian, Chinese, Greek, and Jewish populations (Economidou *et al*, 1985; Ozawa *et al*, 1988; Pitchappan *et al*, 1989; Cao *et al*, 1993), suggesting the disease mutation is an old mutation in tight linkage disequilibrium with HLA-B and -C. Other disease associated alleles, however, such as HLA-Cw7, have also been observed in these populations indicating different haplotypes may be associated with disease in different racial groups. Moreover, linkage disequilibrium between several HLA genes and microsatellites has already been described with the existence of numerous recognizable haplotypes (Foissac *et al*, 1997). Identifying further polymorphic markers within this region and linkage disequilibrium mapping of other ethnic populations with different ancestral haplotypes may lead to better definition of the susceptibility region. We are currently employing this approach in a number of ethnic groups.

Many diseases have been associated with HLA genes, but only a few have predominant class I or class III region associations, including ankylosing spondylitis (HLA-B27) and Behçets disease. It has been suggested that MHC molecules contribute directly to the development of class II associated diseases (such as IDDM) by presenting altered antigen to $CD4^+$ T cells. HLA-B*5701 or HLA-Cw*0602 could be similarly pathogenic in psoriasis through presentation of antigen to $CD8^+$ T cells, but equally they may simply be markers for a nearby gene. The identified region of maximum association, the distal class III and proximal class I regions, contain a variety of other genes important in immune responsiveness, including tumor necrosis factor, lymphotoxin genes, heat shock protein 70, and complement components. MICA and MICB, HLA-like genes which are expressed in epithelial tissues, have recently been strongly associated with Behçets disease (Groh *et al*, 1996; Zwirner *et al*, 1998). In addition, this region includes many nonimmune genes, including some which may be preferentially expressed in the skin. For example the S gene, 160 kbp telomeric to HLA-C, encodes a protein with sequence homology to human keratin 10 (Zhou and Chaplin, 1993), although a single small study has failed to provide evidence that it is associated with susceptibility to psoriasis (Ishihara *et al*, 1996). We have begun characterizing polymorphisms in genes within the candidate region to identify DNA variants associated with psoriasis (Marshall *et al*, 1993; Wei *et al*, 1993; Goldsworthy *et al*, 1997). Establishing a definite MHC locus should also aid in the partitioning of future data, a mechanism likely to be required, in order to define genetic heterogeneity and facilitate the localization of non-MHC loci (Trembath *et al*, 1997).

In conclusion our results confirm both linkage and association of psoriasis to HLA-B, HLA-C, and to the microsatellite marker tn62, suggesting that the susceptibility gene is located in the 285 kb region surrounding the boundary between the HLA class III and I regions. Linkage disequilibrium analysis of other ethnic populations with diverse haplotypes offers the promise of refining this region further with the aim of identifying the 6p21.3 susceptibility locus for psoriasis.

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